Atty Dkt No. 0800-0009.05
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

GREGORY M. PODSAKOFF et al.

Serial No.: 09/755,734 Group Art Unit: 1632

Filing Date: December 4, 1998 Examiner: A. Beckerleg

Title: METHODS FOR DELIVERING DNA TO THE BLOODSTREAM USING RECOMBINANT ADENO-ASSOCIATED VIRUS VIRIONS

DECLARATION OF LINDA B. COUTO, Ph.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Linda B. Couto, Ph.D., hereby declare as follows:

1. I am currently employed by Avigen, Inc., where I hold the title of Director of Applications Research. In this capacity, I direct research and development activities relating to recombinant adeno-associated virus (rAAV)-based gene delivery systems for treating a variety of diseases, including blood disorders such as hemophilia and lysosomal storage disorders such as Gaucher''s disease.

2. I hold a Bachelor of Science (B.S.) degree in Biology from Northeastern University and a Doctorate (Ph.D.) in Molecular Toxicology from the Massachusetts Institute of Technology. I have conducted research in the fields of DNA mutagenesis/repair, virology, and gene therapy. As a post-doctoral fellow at Stanford University, I studied the molecular basis of DNA repair in mammalian cells and lower eukaryotes. For the past 10 years, I have worked in the gene therapy field using both retroviral and adeno-associated viral vectors to treat various diseases. In my position as Director of Applications Research at Avigen, I direct research in gene therapy using

adeno-associated viral based technologies, and have co-authored many scientific publications on this topic. A true and correct copy of my *curriculum vitae* is attached hereto as Exhibit A.

3. I am aware of studies conducted by Avigen collaborators involving intra-arterial administration of AAV virions into rats and dogs. These studies were done to support the filing of an Investigational New Drug Application for administering rAAV virions via the hepatic artery into humans.

4. **Rat Studies.** Nude rats were infused with AAV virions containing the human Factor IX (hF.IX) gene via the tail vein, the portal vein, and the hepatic artery. Serum samples were obtained and analyzed for hF.IX protein. Experimental results indicated that serum levels of circulating hF.IX protein were equivalent between the portal vein and hepatic artery routes of administration and only slightly lower with the tail vein. Data comparing the efficacy of tail vein, portal vein, and hepatic artery administration of AAV vectors is attached hereto as Exhibit B.

5. **Adult Dog Studies.** In large mammalian studies, four adult dogs were injected into the hepatic artery with between 3.7-7.0 x 10^{12} AAV virions per kg. The AAV virions contained the AAV null vector, a vector that contains *E. coli* beta-galactosidase and neomycin phosphotransferase genes with disrupted codons for the initial methionine and no promoters or polyadenylation signals. AAV virion transfer into the liver was evaluated using standard molecular biology techniques. Tests revealed that, in every dog, at least two lobes were transduced by AAV null virions, while all four liver lobes were transduced in two of the dogs. The results of this study are tabulated and attached hereto as Exhibit C.

6. Based on the foregoing studies, it is my professional opinion that AAV virions can be successfully administered via the intra-arterial route and that results obtained therewith are similar, if not identical, to results obtained from intravenous administration of AAV virions.

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11/7/01
Date

Linda B Couto
Linda B. Couto, Ph.D.